Inventor: Wittamer, et al.

Filed: June 25, 2003 Preliminary Amendment

Page 3

The following Listing of the Claims will replace all prior versions and all prior listings of the claims in the present application:

Listing of The Claims:

- 1. (Original) A polypeptide comprising an amino acid sequence N1N2X1X2X3N3X4N4X5, wherein N1, N2, N3, and N4 are aromatic amino acids, and X1-X5 are any amino acids, and wherein the polypeptide binds specifically to a ChemerinR polypeptide.
- 2. (Original) A polypeptide of claim 1, wherein N1 is tyrosine, N2-N4 are phenylalanine.
- 3. (Original) A polypeptide of claim 1, wherein the amino acid sequence is YFX1X2X3FX4FX5, wherein X1-X5 are any amino acid.
- 4. (Original) A polypeptide according to claim 3, wherein X1 is proline, X2, X4, and X5 are selected from group consisting of glycine, alanine, valine, leucine, isoleucine, serine, and threonine, and X3 is either glutamine or asparagine.
- 5. (Original) A polypeptide of claim 1, wherein the amino acid sequence is YFPGOFAFS.
- 6. (Original) A polypeptide of claim 1, wherein the amino acid sequence is QRAGEDPHSFYFPGQFAFS.
- 7. (Original) A polypeptide comprising an amino acid sequence SEQ ID No. 73 (the 157 aa truncated sequence).
- 8. (Original) A polypeptide comprising an amino acid sequence SEQ ID No. 12 (the 143 aa truncated sequence, Prochemerin).
- 9. (Original) A polypeptide comprising an amino acid sequence SEQ ID No. 14 (the 137 aa truncated sequence, Chemerin).
- 10. (Original) The polypeptide in claim 1, wherein the polypeptide is labeled with a moiety selected from the group consisting of a radioisotope, a fluorophore, a quencher of fluorescence, an enzyme, an affinity tag, and an epitope tag.
- 11. (Original) A nucleic acid sequence encoding the amino acid sequence of claim 5.
- 12. (Original) A nucleic acid sequence encoding the amino acid sequence of claim 6.
- 13. (Original) A nucleic acid sequence encoding the amino acid sequence of claim 7.
- 14. (Original) A nucleic acid sequence encoding the amino acid sequence of claim 8.

Inventor: Wittamer, et al.

Filed: June 25, 2003 Preliminary Amendment

- 15. (Original) A nucleic acid sequence encoding the amino acid sequence of claim 9.
- 16. (Original) An expression vector comprising the coding sequence in any one of claims 11-15.
- 17. (Original) An expression vector of claim 16, wherein the expressing vector is a plasmid DNA expression vector.
- 18. (Original) An expression vector of claim 16, wherein the expressing vector is an adenovirus vector comprising the coding sequence under the control of tissue specific, tumor selective promoter.
- 19. (Original) A transgenic animal transfected with an expression vector according to claim 16.
- 20. (Original) A composition comprising the polypeptide in any one of claims 1-10.
- 21. (Original) A composition comprising the nucleic acid sequence in any one of claims 11-15.
- 22. (Original) A therapeutic composition comprising the polypeptide in any one of claims 1-10.
- 23. (Original) A therapeutic composition comprising the nucleic acid sequence in any one of claims 11-15.
- 24. (Original) A method of inhibiting cell proliferation comprising administering to a cell the composition in any one of claims 20.
- 25. (Original) A method of inhibiting cell proliferation comprising administering to a cell the composition in any one of claims 21.
- 26. (Original) A method of inhibiting cell proliferation comprising administering to a cell the expression vector according to claim 16.
- 27. (Original) A method of treating a subject with a disease or disorder comprising administering to the subject a therapeutically effective amount of the composition according claim 22.
- 28. (Original) A method of treating a subject with a disease or disorder comprising administering to the subject a therapeutically effective amount of the composition according claim 23.

Inventor: Wittamer, et al.

Filed: June 25, 2003 Preliminary Amendment

- 29. (Original) A method of treating a subject with a disease or disorder comprising administering to the subject the expression vector according to claim 16.
- 30. (Original) The method according to claim 27, wherein the method is *ex vivo* gene therapy.
- 31. (Original) The method according to claim 28, wherein the method is *ex vivo* gene therapy.
- 32. (Original) The method according to claim 27, wherein the method is *in vivo* gene therapy.
- 33. (Original) The method according to claim 28, wherein the method is *in vivo* gene therapy.
- 34. (Original) A method according to any one of claims 27-33, wherein the disease is selected from the group consisting of: neoplasms located in the: colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital, as well as hypergammaglobulinemia, lymphoproliferative diseases, disorders, and/or conditions, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstron's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease.
- 35. (Original) An antibody that selectively binds to the polypeptide in claim 1.
- 36. (Original) The antibody of claim 35, wherein the antibody is an agonist of Proprechemerin, or Prechemerin, or Chemerin, or analogs or fragments thereof.
- 37. (Original) The antibody of claim 35, wherein the antibody is an antagonist of Proprechemerin, or Prechemerin, or Chemerin, or analogs or fragments thereof.
- 38. (Original) The antibody of claim 35, wherein the antibody inhibits the binding of Proprechemerin, Prechemerin, or Chemerin, or analogs or fragments thereof, to ChemerinR.
- 39. (Original) The antibody of claim 35, wherein the antibody is a monoclonal antibody.
- 40. (Original) The antibody of claim 35, wherein the antibody is a monoclonal antibody that binds to an epitope comprising FSKALPRS.
- 41. (Original) The antibody according to any one of claims 35, wherein the antibody is conjugated or coupled to a detectable label, a radioactive label, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, or a therapeutic agent.

Inventor: Wittamer, et al. Filed: June 25, 2003
Preliminary Amendment

- 42. (Original) The antibody of claim 41, wherein the therapeutic agent is an antimetabolite, an alkylating agent, an antibotic, a growth factor, a cytokine, a cytotoxic agent, a toxin, or an anti-angiogenic agent.
- 43. (Currently Amended) A method of identifying an agent that modulates the interaction between a Chemerin <u>polypeptide</u> and a ChemerinR <u>polypeptide</u>, said method comprising:
- (a) contacting a ChemerinR polypeptide with a Chemerin polypeptide in the presence [and]<u>or</u> absence of a candidate modulator under conditions permitting the binding of said Chemerin polypeptide to said ChemerinR polypeptide; and
- (b) measuring the binding of said ChemerinR polypeptide to said Chemerin polypeptide, wherein a decrease in binding in the presence of said candidate modulator, relative to the binding in the absence of said candidate modulator, identifies said candidate modulator as an agent that modulates the function of ChemerinR polypeptide.
- 44. (Original) The method of claim 43, wherein the ChemerinR polypeptide sequence is SEQ ID No. 2, or 4, or 6, and the Chemerin polypeptide sequence is the polypeptide sequence in claim 1.
- 45. (Currently Amended) The method of claim 43, wherein the ChemerinR polypeptide sequence is SEQ ID No. 2, or 4, or 6, and the Chemerin polypeptide sequence is the polypeptide sequence is SEQ ID No. 14 (Chemerin) or SEQ ID No. 73.
- 46. (Currently Amended) A method of detecting the presence, in a sample, of an agent that modulates the interaction between a Chemerin <u>polypeptide</u> and a ChemerinR polypeptide in a sample, said method comprising:
- (a) contacting a ChemerinR polypeptide with a Chemerin polypeptide in the presence [and]or absence of said sample under conditions permitting the binding of said Chemerin polypeptide to said ChemerinR polypeptide; and
- (b) measuring the binding of said ChemerinR polypeptide to said Chemerin polypeptide, wherein a decrease in binding in the presence of said sample, relative to the binding in the absence of said [candidate modulator]sample, indicates the presence, in said sample of an agent that modulates the function of ChemerinR polypeptide.
- 47. (Original) The method of claim 46, wherein the ChemerinR polypeptide sequence is SEQ ID No: 2, or 4, or 6 and the Chemerin polypeptide sequence is the polypeptide sequence in claim 1.

Inventor: Wittamer, et al.

Filed: June 25, 2003 Preliminary Amendment

- 48. (Currently Amended) The method of claim 46, wherein the ChemerinR polypeptide sequence is SEQ ID No: 2, or 4, or 6 and the Chemerin polypeptide sequence is SEQ ID No. 14 (Chemerin) or SEQ ID No. 73.
- 49. (Currently Amended) A method of identifying an agent that modulates the function of ChemerinR <u>polypeptide</u>, said <u>method</u> comprising:
- (a) contacting a ChemerinR polypeptide with a Chemerin polypeptide in the presence [and]or absence of a candidate modulator; and
- (b) measuring a signaling activity of said ChemerinR polypeptide, wherein a change in the activity in the presence of said candidate modulator relative to the activity in the absence of said candidate modulator identifies said candidate modulator as an agent that modulates the function of [chamerinR]chemerinR polypeptide.
- 50. (Original) The method of claim 49, wherein the ChemerinR polypeptide sequence is SEQ ID No. 2, or 4, or 6, and the Chemerin polypeptide sequence is the polypeptide sequence in claim 1.
- 51. (Currently Amended) The method of claim 49, wherein the ChemerinR polypeptide sequence is SEQ ID No. 2, or 4, or 6, and the Chemerin polypeptide sequence is SEQ ID No. 14 (Chemerin) or SEQ ID No. 73.
- 52. (Currently Amended) A method of identifying an agent that modulates the function of ChemerinR polypeptide, said method comprising:
 - (a) contacting a ChemerinR polypeptide with a candidate modulator;
- (b) measuring a signaling activity of said ChemerinR polypeptide in the presence of said candidate modulator; and
- (c) comparing said activity measured in the presence of said candidate modulator to said activity measured in a [sample]reaction in which said ChemerinR polypeptide is contacted with a Chemerin polypeptide[at its EC₅₀], wherein a difference in said activities is indicative of said candidate modulator [is identified]being [as]an agent that modulates the function of ChemerinR[when the amount of said activity measured in the presence of said candidate modulator is at least 50% of the amount induced by said Chemerin polypeptide present at its EC₅₀].
- 53. (Original) The method of claim 52, wherein the ChemerinR polypeptide sequence is SEQ ID No: 2, or 4, or 6 and the Chemerin polypeptide sequence is the polypeptide sequence in claim 1.

Inventor: Wittamer, et al.

Filed: June 25, 2003 Preliminary Amendment

- 54. (Currently Amended) The method of claim 52, wherein the ChemerinR polypeptide sequence is SEQ ID No: 2, or 4, or 6 and the Chemerin polypeptide sequence is SEQ ID No. 14 (Chemerin) or SEQ ID No. 73.
- 55. (Currently Amended) A method of detecting the presence, in a sample, of an agent that modulates the function of ChemerinR polypeptide, said method comprising:
- (a) contacting a ChemerinR polypeptide with a Chemerin polypeptide in the presence [and]or absence of a candidate modulator;
- (b) measuring a signaling activity of said ChemerinR polypeptide in the presence or absence of said [candidate modulator]sample; and
- (c) comparing the amount of said activity measured in a reaction containing ChemerinR <u>polypeptide</u> and Chemerin polypeptide[s] without said sample to the amount of said activity measured in a reaction [cintaining]containing ChemerinR <u>polypeptide</u>, Chemerin <u>polypeptide</u>, and said sample, wherein a change in said activity in the presence of said sample relative to the activity in the absence of said sample indicates the presence, in said sample, of an agent that modulates the function of ChemerinR <u>polypeptide</u>.
- 56. (Original) The method of claim 55, wherein the ChemerinR polypeptide sequence is SEQ ID No: 2, or 4, or 6 and the Chemerin polypeptide sequence is the polypeptide sequence in claim 1.
- 57. (Currently Amended) The method of claim 55, wherein the ChemerinR polypeptide sequence is SEQ ID No: 2, or 4, or 6 and the Chemerin polypeptide sequence is SEQ ID No. 14 (Chemerin) or SEQ ID No. 73.
- 58. (Currently Amended) A method of detecting the presence, in a sample, of an agent that modulates the function of ChemerinR, said method comprising:
- (a) contacting a ChemerinR polypeptide with [a candidate modulator]said sample;
- (b) measuring a signaling activity of said ChemerinR polypeptide in the presence of said sample; and
- (c) comparing said activity measured in the presence of said sample to said activity measured in reaction in which said ChemerinR polypeptide is contacted with a Chemerin polypeptide[at its EC₅₀], wherein a difference in said activities is indicative of said [candidate modulator is identified as]sample as containing an agent that modulates the function of ChemerinR polypeptide[when the amount of said activity measured in the presence of said

Inventor: Wittamer, et al.

Filed: June 25, 2003 Preliminary Amendment

Page 9

candidate modulator is at least 50% of the [amount of the amount induced by said Chemerin polypeptide present at its EC_{50}].

- 59. (Original) The method of claim 58, wherein the ChemerinR polypeptide sequence is SEQ ID No. 2, or 4, or 6 and the Chemerin polypeptide sequence is the polypeptide sequence in claim 1.
- 60. (Currently Amended) The method of claim 58, wherein the ChemerinR polypeptide sequence is SEQ ID No. 2, or 4, or 6 and the Chemerin polypeptide sequence is SEQ ID No. 14 (Chemerin) or SEQ ID No. 73.

Claims 61-63 (Currently Cancelled).

- 64. (New) The method of any one of claims 43, 46, 49, 52, 55 and 58 wherein said Chemerin polypeptide is detectably labeled.
- 65. (New) A method of diagnosing a disease or disorder characterized by dysregulation of ChemerinR signaling, said method comprising:
- (a) contacting a tissue sample with an antibody specific for a Chemerin polypeptide;
 - (b) detecting binding of said antibody to said tissue sample; and
- (c) comparing the binding detected in step (b) with a standard, wherein a difference in binding relative said standard is diagnostic of a disease or disorder characterized dysregulation of ChemerinR, wherein said Chemerin polypeptide is detectably labeled with a moiety selected from the group consisting of a radioisotope, a fluorophore, a quencher of fluorescence, an enzyme, an affinity tag, and an epitope tag.
- 66. (New) The method of any one of claims 43, 46, 49, 52, 55 and 58 wherein said contacting is performed in or on a cell expressing said ChemerinR polypeptide.
- 67. (New) The method of any one of claims 43, 46, 49, 52, 55 and 58 wherein said contacting is performed in or on synthetic liposomes.

Inventor: Wittamer, et al.

Filed: June 25, 2003 Preliminary Amendment

Page 10

68. (New) The method of any one of claims 43, 46, 49, 52, 55 and 58 wherein said contacting is performed in or on virus-induced budding membranes containing a ChemerinR polypeptide.

- 69. (New) The method of any one of claims 43, 46, 49, 52, 55 and 58 wherein said method is performed using a membrane fraction from cells expressing said ChemerinR polypeptide.
- 70. (New) The method of either of claims 43 or 46 wherein said measuring is performed using a method selected from label displacement, surface plasmon resonance, fluorescence resonance energy transfer, fluorescence quenching, and fluorescence polarization.
- 71. (New) The method of any one of claims 43, 46, 49, 52, 55 and 58 wherein said agent is selected from the group consisting of a peptide, a polypeptide, an antibody or antigen-binding fragment thereof, a lipid, a carbohydrate, a nucleic acid, and a small organic molecule.
- 72. (New) The method of any one of claims 49, 52, 55 and 58 wherein said step of measuring a signaling activity of said ChemerinR polypeptide comprises detecting a change in the level of a second messenger.
- 73. (New) The method of either of claims 49, 52, 55 and 58 wherein the step of measuring a signaling activity comprises measurement of guanine nucleotide binding or exchange, adenylate cyclase activity, cAMP, Protein Kinase C activity, phosphatidylinosotol breakdown, diacylglycerol, inositol triphosphate, intracellular calcium, arachinoid acid, MAP kinase activity, tyrosine kinase activity, or reporter gene expression.
- 74. (New) The method of claim 73 wherein said measuring a signaling activity comprises using an aequorin-based assay.
- 75. (New) A method of modulating the activity of a ChemerinR polypeptide in a cell, said method comprising the step of delivering to said cell an agent that modulates the activity of a ChemerinR polypeptide, such that the activity of ChemerinR is modulated.
- 76. (New) A method of diagnosing a disease or disorder characterized by dysregulation of ChemerinR signaling, said method comprising:

Inventor: Wittamer, et al.

Filed: June 25, 2003 Preliminary Amendment

Page 11

a) contacting a tissue sample with an antibody specific for a ChemerinR polypeptide;

b) detecting binding of said antibody to said tissue sample; and

c) comparing the binding detected in step (b) with a standard, wherein a difference in binding relative to said standard is diagnostic of a disease or disorder characterized by dysregulation of ChemerinR.

77. (New) A method of diagnosing a disease or disorder characterized by dysregulation of ChemerinR signaling, said method comprising:

- a) contacting a tissue sample with an antibody specific for a Chemerin polypeptide;
- b) detecting binding of said antibody to said tissue sample; and
- c) comparing the binding detected in step (b) with a standard, wherein a difference in binding relative to said standard is diagnostic of a disease or disorder characterized by dysregulation of ChemerinR.
- 78. (New) A method of diagnosing a disease or disorder characterized by dysregulation of ChemerinR signaling, said method comprising:
- a) contacting a tissue sample with an antibody specific for a ChemerinR polypeptide and an antibody specific for a Chemerin polypeptide;
 - b) detecting binding of said antibodies to said tissue sample; and
- c) comparing the binding detected in step (b) with a standard, wherein a difference in the binding of either antibody or both, relative to said standard, is diagnostic of a disease or disorder characterized by dysregulation of ChemerinR polypeptide.
- 79. (New) A method of diagnosing a disease or disorder characterized by dysregulation of ChemerinR polypeptide signaling, said method comprising:

Inventor: Wittamer, et al.

Filed: June 25, 2003 Preliminary Amendment

Page 12

a) isolating nucleic acid from a tissue sample;

- b) amplifying a ChemerinR polynucleotide, using said nucleic acid as a template; and
- c) comparing the amount of amplified ChemerinR polynucleotide produced in step (b) with a standard, wherein a difference in said amount of amplified ChemerinR polynucleotide relative to said standard is diagnostic of a disease or disorder characterized by dysregulation of ChemerinR polypeptide.
- 80. (New) A method of diagnosing a disease or disorder characterized by dysregulation of ChemerinR polypeptide signaling, said method comprising:
 - a) isolating nucleic acid from a tissue sample;
 - b) amplifying a ChemerinR polynucleotide, using said nucleic acid as a template; and
- c) comparing the sequence of said amplified ChemerinR polynucleotide produced in step (b) with a standard, wherein a difference in said sequence, relative to said standard is diagnostic of a disease or disorder characterized by dysregulation of ChemerinR polypeptide.
- 81. (New) The method of claim 80, wherein said standard is SEQ ID NO: 1.
- 82. (New) A method of diagnosing a disease or disorder characterized by dysregulation of ChemerinR polypeptide signaling, said method comprising:
 - a) isolating nucleic acid from a tissue sample;
 - b) amplifying a Chemerin polynucleotide, using said nucleic acid as a template; and
- c) comparing the amount of amplified Chemerin polynucleotide produced in step (b) with a standard, wherein a difference in said amount of amplified Chemerin polynucleotide relative to said standard is diagnostic of a disease or disorder characterized by dysregulation of ChemerinR polypeptide.

Inventor: Wittamer, et al.

Filed: June 25, 2003 Preliminary Amendment

Page 13

83. (New) A method of diagnosing a disease or disorder characterized by dysregulation of ChemerinR polypeptide signaling, said method comprising:

- a) isolating nucleic acid from a tissue sample;
- b) amplifying a Chemerin polynucleotide, using said nucleic acid as a template; and
- c) comparing the sequence of said amplified Chemerin polynucleotide produced in step (b) with a standard, wherein a difference in said sequence, relative to said standard is diagnostic of a disease or disorder characterized by dysregulation of ChemerinR.
- 84. (New) The method of claim 80 or claim 83, wherein the step of comparing the sequence comprises minisequencing.
- 85. (New) The method of claim 83, wherein said standard is SEQ ID NO: 7.
- 86. (New) The method of claim 79 or claim 82, wherein said comparing the amount is performed on a microarray.
- 87. (New) The method of claim 80 or claim 83, wherein said comparing the sequence is performed on a microarray.
- 88. The method of claim 52 or 58, wherein said difference in said activities is a 10% increase or decrease of an activity induced by said Chemerin polypeptide.
- 89. The method of claim 52 or 58, wherein said difference in said activities is a 50% increase or decrease of an activity induced by said Chemerin polypeptide.
- 90. (New) A composition comprising an isolated ChemerinR polypeptide and an isolated Chemerin polypeptide.
- 91. (New) An antibody specific for a ChemerinR polypeptide.

Inventor: Wittamer, et al.

Filed: June 25, 2003 Preliminary Amendment

Page 14

92. (New) An antibody specific for a Chemerin polypeptide.

93. (New) A kit for screening for agents that modulate the signaling activity of ChemerinR polypeptide, said kit comprising an isolated ChemerinR polypeptide and packaging materials therefor.

- 94. (New) The kit of claim 90, further comprising a Chemerin polypeptide.
- 95. (New) A kit for screening for agents that modulate the signaling activity of ChemerinR polypeptide, said kit comprising an isolated polynucleotide encoding a ChemR23 polypeptide and packaging materials therefor.
- 96. (New) The kit of claim 95, further comprising an isolated polynucleotide encoding a ChemerinR polypeptide.
- 97. (New) A kit for screening for agents that modulate the signaling activity of ChemerinR, said kit comprising a cell transformed with a polynucleotide encoding a ChemerinR polypeptide and packaging materials therefor.
- 98. (New) A kit for the diagnosis of a disease or disorder characterized by dysregulation of ChemerinR polypeptide signaling, said kit comprising an isolated ChemerinR polypeptide and packaging materials therefor.
- 99. (New) The kit of claim 98, further comprising a Chemerin polypeptide.
- 100. (New) A kit for the diagnosis of a disease or disorder characterized by dysregulation of ChemerinR polypeptide signaling, said kit comprising an isolated polynucleotide encoding a ChemerinR polypeptide and packaging materials therefor.
- 101. (New) The kit of claim 100, further comprising an isolated polynucleotide encoding a Chemerin polypeptide.

Inventor: Wittamer, et al.

Filed: June 25, 2003 Preliminary Amendment

Page 15

102. (New) A kit for the diagnosis of a disease or disorder characterized by dysregulation of ChemerinR polypeptide signaling, said kit comprising a cell transformed with a polynucleotide encoding a ChemerinR polypeptide and packaging materials therefor.

- 103. (New) A non-human mammal having a homozygous null mutation in the gene encoding ChemerinR polypeptide.
- 104. (New) A non-human mammal transgenic for a ChemerinR polynucleotide.
- 105. (New) A non-human mammal transgenic for a Chemerin polynucleotide.